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(54) Title: <b>PHARMACOLOGIC DRUG COMBINATION IN VAGAL-INDUCED ASYSTOLE</b>  (57) Abstract  Controlled cessation of heart beat during coronary bypass surgery and other cardiac surgeries on a beating heart improves surgical technique, and is achieved typically by electrical stimulation of the vagus nerve and administration of a combination of drugs.		

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## PHARMACOLOGIC DRUG COMBINATION IN VAGAL-INDUCED ASYSTOLE

### Background Of The Invention

5 Minimally invasive direct coronary artery bypass (MIDCAB) surgery, both via sternotomy and alternative incisions, is a substantially revolutionary development in surgery for allowing bypass surgery to be conducted on a beating heart. However, beating heart surgery shows an undesirably higher rate of early graft failure than conventional coronary artery bypass procedures using  
10 cardiopulmonary bypass and cardioplegia. The technical difficulty of sewing the coronary artery anastomosis on a beating heart is likely an important factor in this difference in outcome between the two techniques. Controlled intermittent asystole (CIA) during brief intervals required for placing anastomotic sutures is suitable for improving the precision of coronary anastomoses performed on a beating heart and  
15 reducing graft failure while increasing ease of operation.

Cardiopulmonary bypass (CPB) and chemical arrest using cardioplegia solutions have traditionally provided surgeons with optimal operative conditions: hemodynamic control and cardiac quiescence. This optimal field has contributed to technical success in increasingly complex cardiac surgical operations. However,  
20 there has been recent interest in performing coronary artery bypass surgery without either complete cardiopulmonary bypass or cardioplegia. The quality of the distal anastomosis is a primary concern among cardiac surgeons who observe and perform coronary artery bypass graft (CABG) procedures unaided by cardioplegic arrest and cardiopulmonary bypass. Coronary artery bypass graft failure rates  
25 reported with minimally invasive direct coronary artery bypass range from 3.8 to 8.9%, while traditional CABG on CPB has a reported anastomotic failure rate of 0.12%. This may reflect a difference in anastomotic precision between MIDCAB and CPB-aided CABG. Although the benefits of avoiding extracorporeal circulation and global cardioplegia in beating heart procedures are important, they  
30 do not outweigh the performance of an optimal coronary anastomosis.

The key difference in the anastomotic results between conventional CABG and beating heart CABG is related to achieving elective asystole during construction of the distal anastomosis. Cardiac motion can be minimized during MIDCAB procedures via pharmacologic bradycardia (adenosine,  $\beta$  blockade) and  
35 mechanical stabilization using various devices. Although these techniques do improve operative conditions, they only approximate the advantages of elective asystole achieved with CPB and cardioplegia.

Applicants show that a state of controlled intermittent asystole (CIA) is produced off CPB, which provides a major advantage otherwise gained by cardioplegic arrest on CPB. In particular, CIA is achieved using unilateral (or bilateral) vagus nerve stimulation coupled with pharmacologic suppression of electromechanical escape activity.

Applicants demonstrate that elective, controlled intermittent asystole is produced by vagus nerve stimulation after treatment with an acetylcholinesterase inhibitor, a  $\beta$ -adrenergic receptor blocker, or a calcium channel blocker, or combinations thereof.

10

#### Detailed Description of the Figures

**Figure 1** Duration of asystole achieved during 60 second vagal stimulation. Lines connect the periods of asystole observed in the non-drug treated and drug treated states in each experimental animal. Drug administration lengthened significantly the period of asystole.

15

**Figure 2.** Representative left ventricular and aortic pressure tracings during 60 second vagal stimulation in the non-drug treated (A) and drug treated states (B). Dark and open arrows mark the initiation and termination of the vagal impulse, respectively. Before drug treatment, a short pause followed by escape and bradycardia was observed during the 60 second impulse. After drug treatment, prolonged asystole occurred during the 60 second impulse with return of mechanical function after termination. lvp - left ventricular pressure; aop - aortic pressure.

20

**Figure 3.** Representative left ventricular and aortic pressure tracings during sequential 15 second vagal stimulations in the non-drug treated (A) and drug treated states (B). Dark and open arrows mark the initiation and termination of the vagal impulses, respectively. Before drug treatment, each 15 second stimulation produced a short pause followed by bradycardia, while after drug treatment, asystole lasted the duration of each 15 second stimulation. lvp - left ventricular pressure; aop - aortic pressure.

25

#### Abbreviations and Definitions

CABG	Coronary artery bypass graft
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CIA	Controlled intermittent asystole
CPB	Cardiopulmonary bypass
MIDCAB	Minimally invasive direct coronary artery bypass; intended to include any CABG without the use of global cardioplegia; synonymous with beating heart surgery, irrespective of incision

#### **Detailed Description Of The Invention**

5 Increased acetylcholine activity by acetylcholinesterase inhibition and prevention of electromechanical escape activity by  $\beta$ -adrenergic receptor and calcium channel blockade during vagal stimulation produces a marked potentiation of vagal-induced asystole and a means of achieving CIA. CIA achieved by pharmacologic potentiation of vagal-induced asystole is a suitable technique to facilitate MIDCAB operations. In particular, anastomoses and other complex  
10 suturing is facilitated during such controlled asystolic events, a readily appreciated advantage in surgery involving minimally invasive direct coronary artery bypass operations on a beating heart. CIA might have particular advantages in partially or totally endoscopic CABG, and possibly in percutaneous or surgical transmyocardial laser revascularization.

15 The present invention provides a pharmaceutical composition, comprising an acetylcholinesterase inhibitor, a  $\beta$ -adrenergic receptor blocker, and a calcium channel blocker, said composition useful for performing beating heart surgery. The invention also provides that the composition is useful for controlled intermittent asystole in minimally invasive direct coronary artery bypass surgery.  
20 The invention further provides that the compositions can be administered in combination with vagus nerve stimulation. Vagus nerve stimulation can be achieved by direct or indirect electrical stimulation.

In preferred independent embodiments, the acetylcholinesterase inhibitor can be pyridostygmine bromide, the  $\beta$ -adrenergic receptor blocker can be

propranolol hydrochloride, and the calcium channel blocker can be verapamil bromide.

The invention also provides a pharmaceutical composition, comprising an acetylcholinesterase inhibitor and a  $\beta$ -adrenergic receptor blocker, said composition  
5 useful for performing beating heart surgery. In preferred embodiments, the acetylcholinesterase inhibitor can be pyridostygmine bromide, the  $\beta$ -adrenergic receptor blocker can be propranolol hydrochloride, and the calcium channel blocker can be verapamil bromide. The invention also provides that the composition is useful for controlled intermittent asystole in minimally invasive  
10 direct coronary artery bypass surgery. The invention further provides that the compositions can be administered in combination with vagus nerve stimulation. Vagus nerve stimulation can be achieved by direct or indirect electrical stimulation.

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20 direct coronary artery bypass surgery. The invention further provides that the compositions can be administered in combination with vagus nerve stimulation. Vagus nerve stimulation can be achieved by direct or indirect electrical stimulation.

The principal challenge of beating heart CABG surgery has been to recreate the advantageous operative conditions of a quiescent operative field provided  
25 during conventional CABG with CPB and cardioplegic arrest. A variety of pharmacologic manipulations and mechanical stabilizing techniques assist in performing CABG off pump. These interventions to date minimize, but do not eliminate, cardiac motion. The concept that a state of controlled intermittent asystole improves the conditions for construction of distal coronary artery bypass  
30 anastomosis in non-CPB assisted cases was demonstrated by applicant. CIA is defined as operator-initiated and controlled intervals of mechanical cardiac standstill. These intervals may be timed to coincide with placement of sutures in the anastomosis, after which normal cardiac rhythm and hemodynamics are restored while preparations are made for the next successive stitch. Experiments  
35 reported by the applicant indicate that the minor bradycardia known to be produced by vagus nerve stimulation is dramatically augmented to function as an electromechanical "on-off switch" by pharmacological inhibition of

acetylcholinesterase and blockade of  $\beta$ -adrenergic receptors and calcium channels. Controlled intermittent asystole may prove equally useful for CPB-assisted cardiac surgery without global cardioplegia.

5 The chronotropic effects of vagal nerve stimulation have been well described and typically produce an initial pause followed by a "vagal escape" beat and sustained bradycardia during continuous optimal stimulation of the vagus nerve. Cardiac responses to a 60 second vagal stimulation without adjunctive therapy achieved an average pause of 1.6 seconds terminated by vagal escape beats with a 19% reduction in heart rate. Vagus nerve stimulation alone did not produce  
10 a controlled period of asystole desired for CIA. In contrast, a triple pharmacologic regimen of e.g., pyridostigmine, propranolol and verapamil inhibited vagal escape, and allowed sustained periods of asystole lasting up to 60 seconds and sequential asystoles of 15 seconds each. Segmental asystoles had no significant hemodynamic consequences.

15 It is apparent that suppression of the electromechanical escape during vagal stimulation is necessary to produce a sufficient interval of asystole to allow during which a single stitch may be reliably placed during construction of a distal CABG anastomosis. The negative chronotropic effects of vagal stimulation are produced by acetylcholine release. Acetylcholine activity may be enhanced by inhibition of  
20 acetylcholinesterase activity by agents such as pyridostigmine. Additionally, it is known that calcium channel blockade by e.g. verapamil potentiates the negative chronotropic effect of vagus nerve stimulation. Another component in electromechanical escape may be related to increased catecholamine activity in the sympathetic nervous system, triggered by hypotension. Catecholamines increase  
25 the rate of diastolic depolarization and decrease the threshold potential.  $\beta$ -adrenergic receptor blockade via e.g. propranolol reduces the effects of catecholamine activity and facilitates suppression of electromechanical escape.

Administration of this combination therapy produced a significant reduction in heart rate and maximum developed ventricular pressure along with an increase in  
30 left ventricular end-diastolic pressure, but did not alter mean arterial pressure. There was no apparent fatigue of this pharmacologic effect after sequential stimulations. The animals used for pilot experiments appeared to tolerate this pharmacologic regimen without other adverse hemodynamic side effects, such as acidosis.

35 The short-term hemodynamic effects of a single prolonged stimulation were found to be substantially insignificant. Likewise the metabolic consequences as detected by pH and changes in base deficit were insignificant.

The pharmacologic regimen used in this investigation sustained the period of vagal-induced asystole for about sixty minutes. This interval would allow more than sufficient time for construction of a distal CABG anastomosis. Animals followed for two hours after administration of drugs displayed responses to vagal stimulation similar to those in the non-drug treated state, confirming reversibility of the drug effects.

An untoward effect of the pharmacologic regimen which requires consideration before clinical application is vagal-induced secretions. All animals displayed significant salivation after initiation of vagal stimulation. However, there were no problems with oxygenation and ventilation due to tracheobronchial secretions in these experiments. Vagal-induced oropharyngeal and tracheobronchial secretions are pertinent in the clinical setting. Additionally, the effects on recurrent laryngeal nerve function require consideration.

Evidence suggests that the long-term effects of this regimen on the vagus nerve are not harmful. Chronic vagus nerve stimulation has been utilized as therapy for intractable seizure disorders without apparent nerve injury or impaired function. Applicants have shown that vagal-mediated chronotropic control at two hours after completion of the experimental protocol was similar to the non-drug treated state.

In summary, controlled intermittent asystole can be achieved by potentiation of vagal-induced asystole via a pharmacologic combination of e.g., propranolol and verapamil for suppression of electromechanical escape and e.g., pyridostigmine for acetylcholinesterase inhibition. Asystole can be reproducibly achieved for prolonged intervals and for shorter multiple sequential intervals using this technique.

### Nerve Stimulation

To achieve consistent asystole, applicants have found that nerve stimulation of the right vagus nerve before or after treatment with the pharmacological combinations of the present invention is preferred.

Electrical stimulation is carried out on the right vagus nerve, preferably at a site on the neck. Other suitable locations for vagus nerve stimulation include, but are not limited to, unipolar or bipolar electrical stimulation of the right or left vagus, or both, stimulation of the vagus in the chest after sternotomy, stimulation with a percutaneous catheter or electrode probe in the internal jugular vein, esophagus, or trachea, or combination of these. The nerve stimulator is typically a Grass wire with a single point of contact, but other suitable stimulators include a



pair of pacing wires or electrodes placed about 1 cm apart to allow bipolar prodromic stimulation. A single continuous impulse is applied of between about 5 seconds to about 90 seconds, preferably between about 5 seconds and about 15 seconds to allow single stitch during surgery. Impulse parameters can readily be varied, e.g., a frequency range of between about 1Hz and about 500Hz, preferably between about 20 Hz to about 80Hz, more preferably about 40 Hz, with an amplitude between about 1 to about 40 volts.

#### Pharmacologic Potentiation

10 The acetylcholinesterase inhibitor is also known as a cholinesterase inhibitor. Suitable acetylcholinesterase inhibitors include, but are not limited to tacrine hydrochloride, pyridostigmine bromide, neostigmine methylsulfate, and edrophonium chloride. One preferred acetylcholinesterase inhibitor is pyridostigmine bromide. Acetylcholinesterase inhibitors are administered in a dosage range between about 0.01 mg/kg and about 100 mg/kg, preferably between about 0.1 mg/kg and about 2.0 mg/kg, more preferably about 0.5 mg/kg.

The beta-adrenergic receptor blocker is also known as a beta-adrenergic blocking agent. Suitable beta-adrenergic receptor blockers include, but are not limited to, sotalol HCl, timolol maleate, esmolol hydrochloride, carteolol hydrochloride, propranolol hydrochloride, betaxolol hydrochloride, penbutolol sulfate, metoprolol tartrate, acetbutolol hydrochloride, the combination of atenolol and chlorthalidone, metoprolol succinate, pindolol, and bisoprolol fumarate. One preferred beta-adrenergic receptor blocker is propranolol hydrochloride. Beta-adrenergic receptor blockers are administered in a dosage range between about 0.01 mg/kg and about 100 mg/kg, preferably between about 0.1 mg/kg and about 2.0 mg/kg, more preferably about 80 µg/kg.

Suitable calcium channel blockers include, but are not limited to, nifedipine, nicardipine hydrochloride, diltiazem HCl, isradipine, verapamil hydrochloride, nimodipine, amlodipine besylate, felodipine, bepridil hydrochloride, and nisoldipine. One preferred calcium channel blocker is verapamil hydrochloride. Calcium channel blockers are administered in a dosage range of between about 1 mg/kg to about 1000 mg/kg, preferably between about 10 mg/kg and about 200 mg/kg, more preferably about 50 µg/kg.

It will be understood that other dosage combinations may be effective. The appropriate dosage is determined by the age, weight, sex, health status of the patient, and may vary with a variety of other factors according to conventional clinical practice.

**EXAMPLE 1****Experimental Preparation**

5 The sheep in the examples of the present invention received humane care in compliance with "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1985). The experimental protocol was approved by the Institutional Animal Care and Use  
10 Committee of Emory University.

Seven sheep weighing 44 to 45 kg were premedicated with xylazine (0.1mg/kg) and atropine (0.2 mg/kg) 30 minutes prior to induction of anesthesia with intravenous thiopental (2.2 mg/kg) and lidocaine (2.2 mg/kg). The animals were endotracheally intubated and placed on a volume ventilator with isoflurane for  
15 maintenance of anesthesia. Limb leads and precordial lead were placed for electrocardiographic monitoring. The right femoral artery was cannulated for arterial pressure and arterial blood gas monitoring. Tidal volume was adjusted to 10 cc/kg and a rate of 12 breaths per minute, with adjustments made to maintain pH at 7.35-7.45, pO<sub>2</sub> greater than 100mm Hg, and pCO<sub>2</sub> between 35-45 mmHg.

20 A right cervical incision was performed, the vagus nerve was carefully isolated and a nerve stimulation probe (Harvard Apparatus, South Natick, MA) was placed on the nerve. A median sternotomy was made to expose the heart. A high-fidelity solid-state micromanometer (Millar Inc., Houston, TX) was secured in the ascending aorta for aortic blood pressure monitoring. An additional  
25 micromanometer was introduced into the left ventricle through the apex for left ventricular pressure monitoring.

**EXAMPLE 2****Experimental Protocol**

30 Each animal underwent vagal stimulation before and after drug administration. The pharmacologic regimen consisted of pyridostigmine (0.5 mg/kg) for acetylcholinesterase inhibition, propranolol (80 µg/kg) for β-adrenergic receptor blockade, and verapamil (50 µg/kg) for calcium channel blockade. Vagal stimulation was performed with a nerve stimulator (Grass Instrument Co., Quincy,  
35 MA) in the monopolar mode at a frequency of 40 Hz, an impulse duration of 0.4 msec, and an amplitude of 2-6 volts. Vagal stimulations were delivered in two regiments: 1) continuous 60 second impulse and 2) sequential 15 second

- impulses. The continuous 60 second stimulation was designed to determine the longevity of vagal-induced asystole and the physiologic effects of prolonged vagal-induced hypotension. Sequential 15 second vagal stimulations were performed to simulate the suturing intervals required for graft anastomoses and to determine whether cardiac fatigue, electromechanical escape, and physiologic effects occurred under these practical conditions.

### **EXAMPLE 3**

#### **Data Acquisition and Analysis**

- Electrocardiographic and hemodynamic data were gathered via an analog-to-digital conversion board (Data Translation, Inc., Marlboro, MA) and processed, stored, and analyzed via a microprocessor personal 486 computer (Compaq Computer Corp., Houston, TX) using interactive proprietary software (Spectrum™, Triton Technology, San Diego, CA). The system was configured to collect 4 channels of physiologic data at a frequency of 50 Hz (sufficient for slow-wave waveforms and mean pressure data) over a 200 second period that encompassed the 60 second stimulation or the sequential 15 second train of stimulations. The software allowed subsequent videographic display and analysis of the hemodynamic data.

### **EXAMPLE 4**

#### **Results**

- Before drug administration, vagal stimulation for 60 seconds produced a brief pause in electromechanical activity ( $1.6 \pm 0.9$  seconds) followed by vagal escape and resumption of sinus rhythm with a reduction in heart rate by  $19.4 \pm 11.9\%$  compared to pre-stimulation heart rate. Similarly, sequential 15 second vagal stimulation performed to stimulate the suturing intervals required for CABG anastomoses produced a short pause ( $1.1 \pm 0.4$  seconds) followed by vagal escape and sinus rhythm with a reduction in heart rate of  $37 \pm 6\%$ .

- Administration of the pharmacologic regimen (propranolol, verapamil, pyridostigmine) reduced the heart rate and increased the left ventricular end diastolic pressure, but did not affect the mean arterial pressure or maximum dP/dt as shown in Table 1.

**Table 1. Hemodynamics before and after drug treatment**

	Before drugs (mean $\pm$ SEM)	After drugs (mean $\pm$ SEM)	p value (paired t test)
Heart rate (bpm)	114 $\pm$ 4	87 $\pm$ 4	0.002
MAP (mm Hg)	84 $\pm$ 5	84 $\pm$ 5	NS
dP/dt max (mm' Hg/sec)	3286 $\pm$ 232	2847 $\pm$ 140	NS
LVEDP (mm HG)	3.9 $\pm$ 0.5	7.3 $\pm$ 0.9	0.005

bpm - beats per minute; dP/dt max - maximum developed left ventricular pressure;  
LVEDP - left ventricular end diastolic pressure; MAP - mean aortic pressure; NS -  
not significant; SEM - standard error of the mean; sec - seconds.

5

After drug administration, 60 second vagal stimulation produced asystole averaging  $52 \pm 5.6$  seconds. The individual responses of the animals before and after drug administration are shown in Figure 1. Six animals achieved controlled asystole. Five of these six achieved controlled asystole for greater than 50 seconds. The effects of 60 second vagal stimulation before and after drug treatment in responsive animals are contrasted by representative left ventricular and aortic pressure tracings are shown for a representative experiment in Figure 2. Before drug regimen treated, vagal stimulation produced no appreciable change in cardiac rhythm or hemodynamics. In contrast, the triple drug regimen facilitated a consistent asystole and circulatory arrest until the stimulus was withdrawn, after which hemodynamics were rapidly restored to pre-stimulation values. The prolonged asystole and circulatory arrest produced no significant differences in the hemodynamic parameters measured before and after drug-aided 60 second vagal stimulation (Table 2).

10

15

**Table 2. Hemodynamics pre- and post-asystole produced by 60 second stimulation after drug treatment**

	Pre-asystole (mean $\pm$ SEM)	Post-asystole (mean $\pm$ SEM)	p value (paired t test)
Heart rate bpm)	91 $\pm$ 8	87 $\pm$ 7	NS
MAP (mm Hg)	86 $\pm$ 6	92 $\pm$ 6	NS
dP/dt max (mm Hg/sec)	3032 $\pm$ 182	3223 $\pm$ 212	NS
LVEDP(mmHg)	5.8 $\pm$ 1.0	6.0 $\pm$ 0.8	NS

bpm - beats per minute; dP/dt max - maximum developed left ventricular pressure;

- 5 LVEDP - left ventricular end diastolic pressure; MAP - mean aortic pressure; NS - not significant; SEM - standard error of the mean; sec - seconds.

Likewise there was no difference in the parameters measured by arterial blood gases at one and five minutes after the 60 second stimulation compared to pre-stimulation values (Table 3).

10

**Table 3. Arterial blood gas data pre-, 1 minute post-, and 5 minutes post-systole produced by 60 second stimulation after drug treatment**

	Pre-asystole (mean $\pm$ SEM)	Post-asystole 1 minute (mean $\pm$ SEM)	5 minutes (mean $\pm$ SEM)	p p value (ANOVA)
pH	7.42 $\pm$ 0.03	7.40 $\pm$ 0.03	7.42 $\pm$ 0.03	NS
PCO <sub>2</sub> (mm Hg)	41 $\pm$ 4	42 $\pm$ 4	40 $\pm$ 4	NS
PO <sub>2</sub> (mm Hg)	377 $\pm$ 87	380 $\pm$ 75	390 $\pm$ 83	NS
HCO <sub>3</sub> (mEq/L)	26 $\pm$ 1	26 $\pm$ 1	26 $\pm$ 1	NS
Base excess (mEq/L)	1.2 $\pm$ 0.7	1.0 $\pm$ 0.4	1.3 $\pm$ 0.5	NS

- 15 ANOVA - one-way analysis of variance with repeated measures; NS - not significant; SEM - standard error of the mean.

Five to six sequential 15 second vagal stimulations in the drug treated state produced consistent and stable asystole (Figure 3). Three of the six animals had a single escape beat during one of the 15 second stimulations. The other three displayed complete asystole during each of the 15 second stimulations. A  
5 sustained cardiac rhythm began an average of  $5.3 \pm 1.8$  seconds after termination of each 15 second impulse during which interval a single beat was often observed immediately after withdrawal of stimulation.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be  
10 understood that the practice of the invention encompasses all of the usual variations, adaptations, and modifications, as come within the scope of the following claims and its equivalents.

## CLAIMS

What Is Claimed Is:

1. A pharmaceutical composition, comprising an acetylcholinesterase inhibitor, a  $\beta$ -adrenergic receptor blocker, and a calcium channel blocker, said composition useful for performing heart surgery.
2. The composition of claim 1, useful for controlled intermittent asystole in minimally invasive direct coronary artery bypass surgery.
3. The composition of claim 1, wherein said composition is administered in combination with vagus nerve stimulation, accompanied by direct application of at least one unipolar or bipolar electrode to either or both vagus nerves or by placement of at least one unipolar or bipolar electrode in the trachea, esophagus or internal jugular vein, or some combination thereof.
4. The composition of claim 1, wherein the acetylcholinesterase inhibitor is pyridostygmine bromide.
5. The composition of claim 4, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.01 mg/kg and about 100.0 mg/kg.
6. The composition of claim 5, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.1 mg/kg and about 2.0 mg/kg.
7. The composition of claim 1, wherein the  $\beta$ -adrenergic receptor blocker is propranolol hydrochloride.
8. The composition of claim 7, wherein the  $\beta$ -adrenergic receptor blocker is in a dosage range between about 0.01 mg/kg and about 100.0 mg/kg.
9. The composition of claim 8, wherein the  $\beta$ -adrenergic receptor blocker is in a dosage range between about 0.1 mg/kg and about 2.0 mg/kg.
10. The composition of claim 1, wherein the calcium channel blocker is verapamil bromide.

11. The composition of claim 10, wherein the calcium channel blocker is in a dosage range between about 1 mg/kg and about 1000 mg/kg.
12. The composition of claim 11, wherein the calcium channel blocker is in a dosage range between about 10 mg/kg and about 200 mg/kg.
13. A pharmaceutical composition, comprising the acetylcholinesterase inhibitor pyridostigmine bromide, the  $\beta$ -adrenergic receptor blocker propranolol hydrochloride, and the calcium channel blocker verapamil bromide, said composition useful for controlled intermittent asystole in minimally invasive direct coronary artery bypass heart surgery.
14. The composition of claim 13, wherein said composition is administered in combination with vagus nerve stimulation, accompanied by direct application of at least one unipolar or bipolar electrode to either or both vagus nerves or by placement of at least one unipolar or bipolar electrode in the trachea, esophagus or internal jugular vein, or some combination thereof.
15. The composition of claim 13, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.01 mg/kg and about 100.0 mg/kg, wherein the beta-adrenergic receptor blocker is in a dosage range between about 0.01 mg/kg and about 100.0 mg/kg, and wherein the calcium channel blocker is in a dosage range between about 1 mg/kg and about 1000 mg/kg.
16. The composition of claim 15, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.1 mg/kg and about 2.0 mg/kg, wherein the beta-adrenergic receptor blocker is in a dosage range between about 0.1 mg/kg and about 2.0 mg/kg, and wherein the calcium channel blocker is in a dosage range between about 10 mg/kg and about 200 mg/kg.
17. A pharmaceutical composition, comprising an acetylcholinesterase inhibitor and a  $\beta$ -adrenergic receptor blocker, said composition useful for performing heart surgery.
18. The composition of claim 17, wherein said composition is administered in combination with vagus nerve stimulation, accompanied by direct application of at least one unipolar or bipolar electrode to either or both vagus nerves or by



placement of at least one unipolar or bipolar electrode in the trachea, esophagus or internal jugular vein, or some combination thereof.

19. The composition of claim 17, wherein the acetylcholinesterase inhibitor is pyridostygmine bromide.

20. The composition of claim 19, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.01 mg/kg and about 100.0 mg/kg.

21. The composition of claim 20, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.1 mg/kg and about 2.0 mg/kg.

22. The composition of claim 17, wherein the  $\beta$ -adrenergic receptor blocker is propranolol hydrochloride.

23. The composition of claim 22, wherein the  $\beta$ -adrenergic receptor blocker is in a dosage range between about 0.01 mg/kg and about 100.0 mg/kg.

24. The composition of claim 23, wherein the  $\beta$ -adrenergic receptor blocker is in a dosage range between about 0.1 mg/kg and about 2.0 mg/kg.

25. A pharmaceutical composition, comprising an acetylcholinesterase inhibitor and a calcium channel blocker, said composition useful for performing heart surgery.

26. The composition of claim 25, wherein said composition is administered in combination with vagus nerve stimulation, accompanied by direct application of at least one unipolar or bipolar electrode to either or both vagus nerves or by placement of at least one unipolar or bipolar electrode in the trachea, esophagus or internal jugular vein, or some combination thereof.

27. The composition of claim 25, wherein the acetylcholinesterase inhibitor is pyridostygmine bromide.

28. The composition of claim 27, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.01 mg/kg and about 100.0 mg/kg.

29. The composition of claim 28, wherein the acetylcholinesterase inhibitor is in a dosage range between about 0.1 mg/kg and about 2.0 mg/kg.
30. The composition of claim 25, wherein the calcium channel blocker is verapamil bromide.
31. The composition of claim 30, wherein the calcium channel blocker is in a dosage range between about 1 mg/kg and about 1000 mg/kg.
32. The composition of claim 31, wherein the calcium channel blocker is in a dosage range between about 10 mg/kg and about 200 mg/kg.

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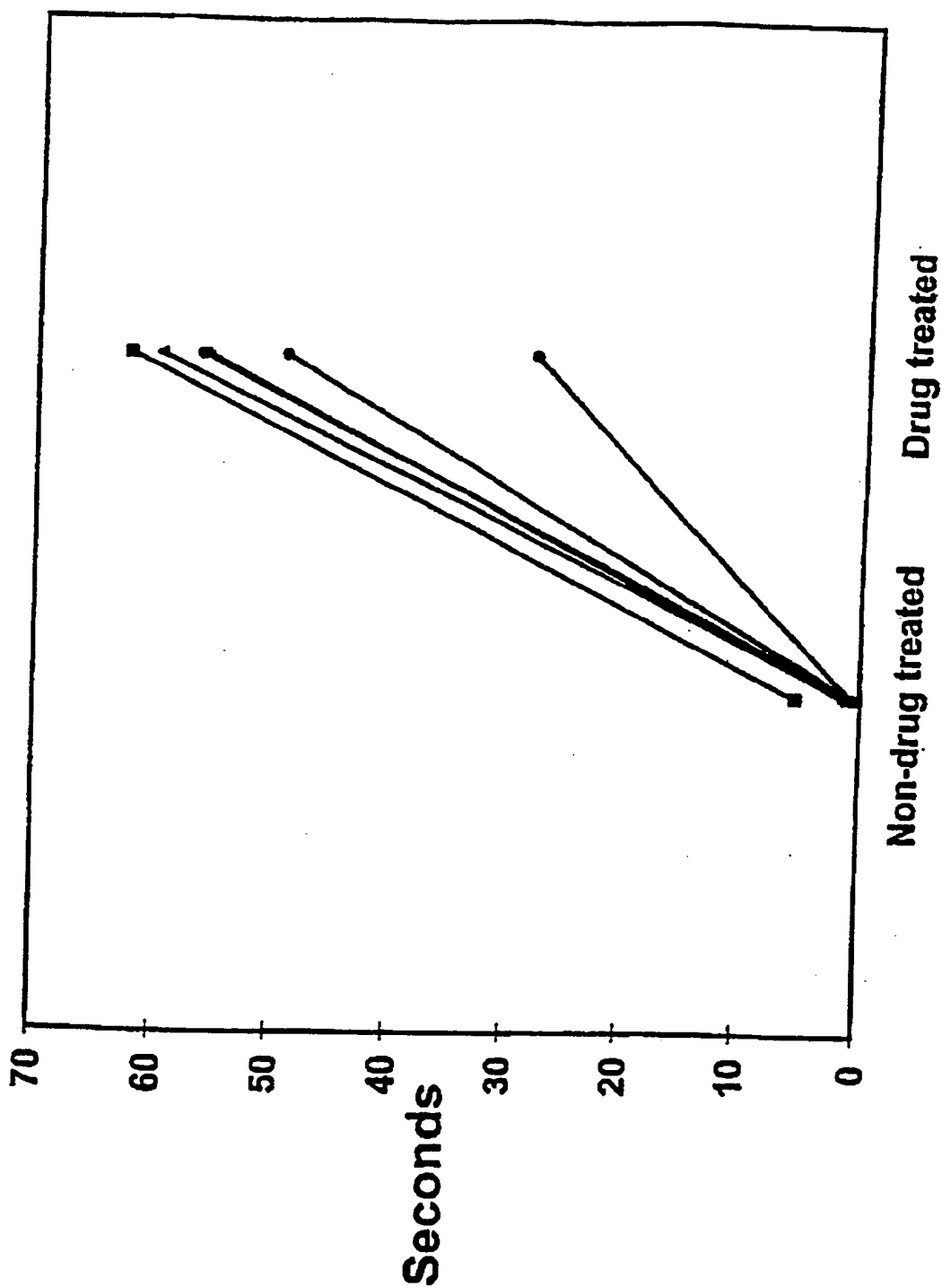


FIG. 1

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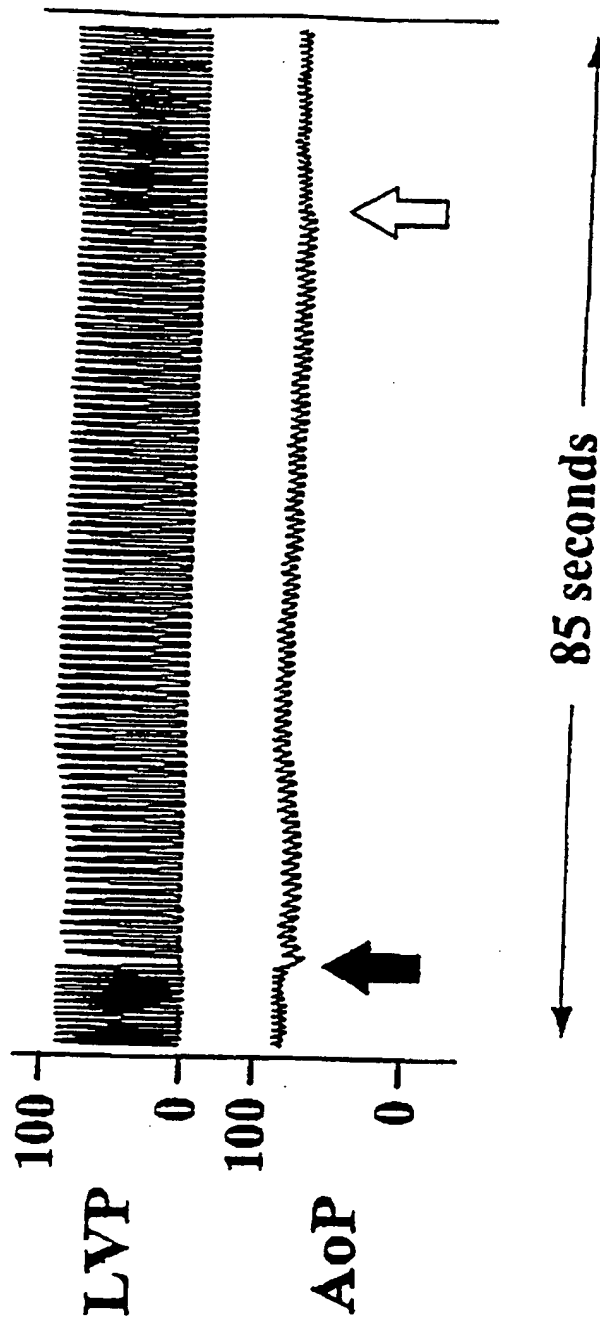


FIG. 2 A

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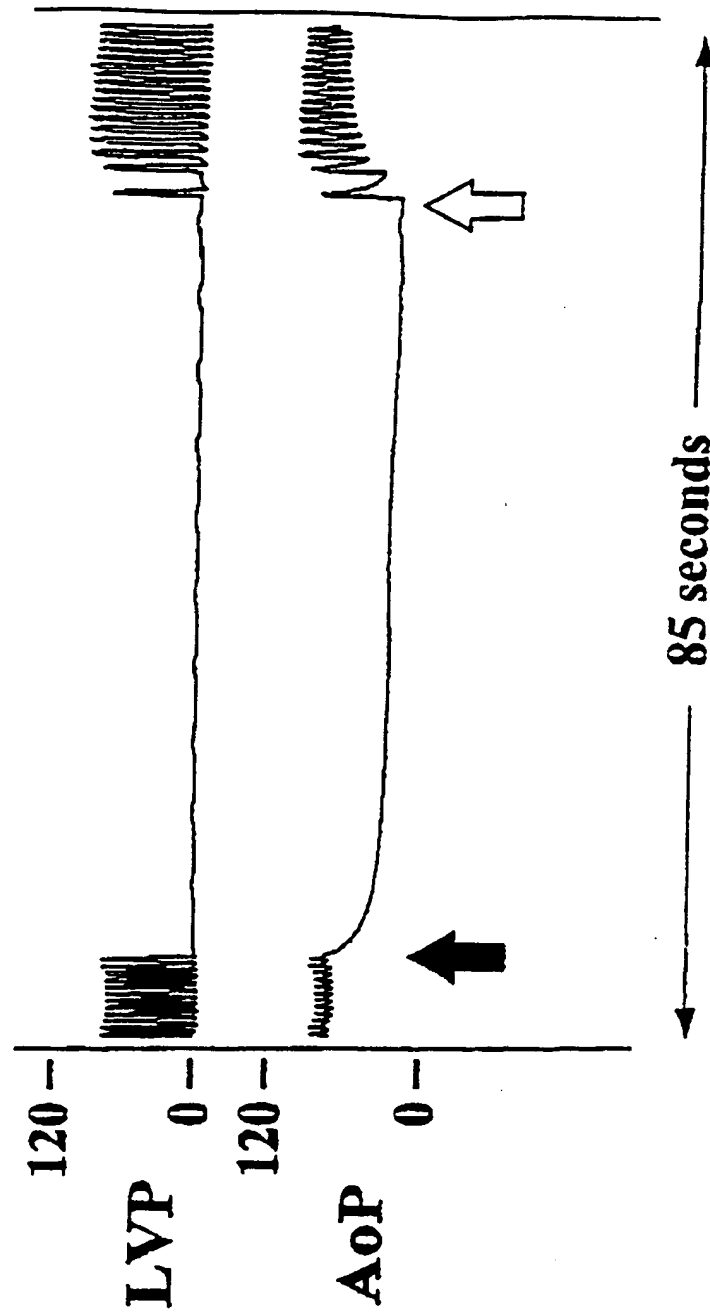


FIG. 2 B

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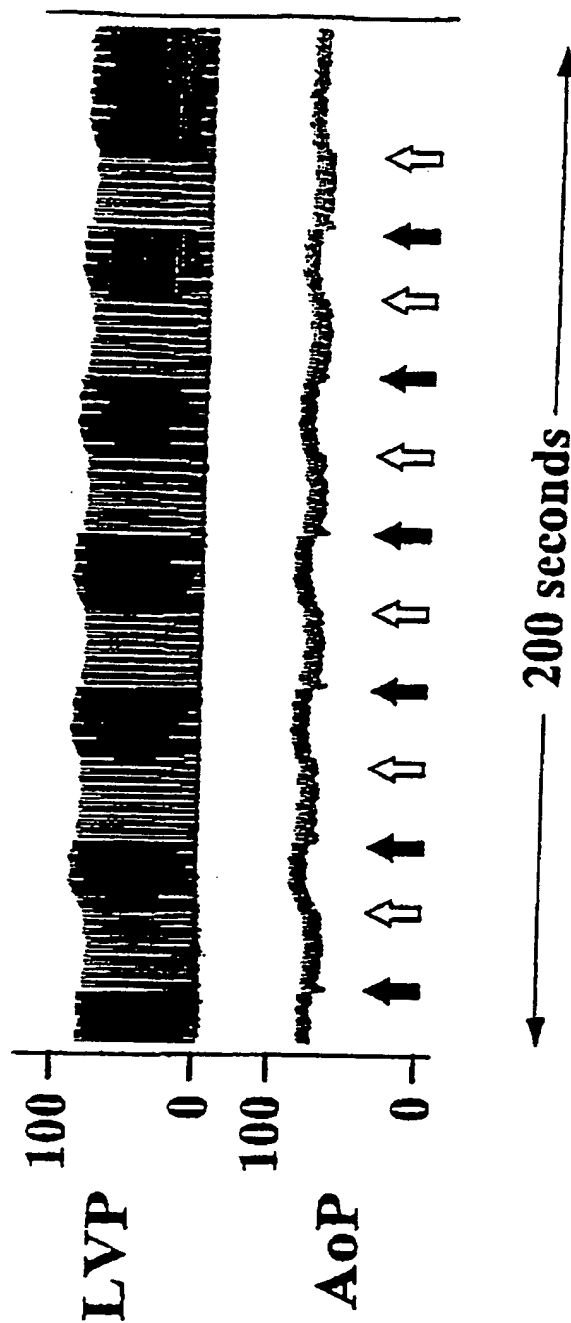


FIG. 3 A

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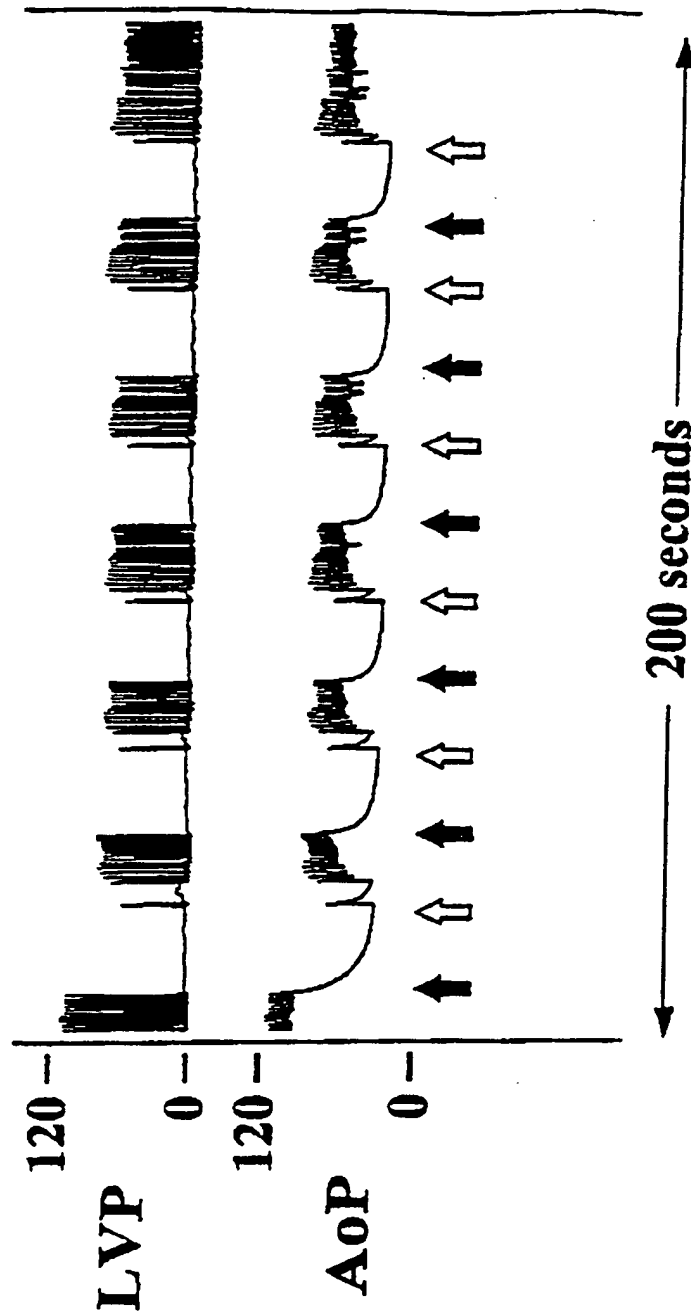
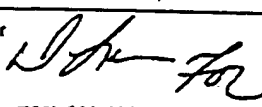


FIG. 3 B

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/17630

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(6) :A61K 31/27, 31/135 US CL :514/479, 484, 652, 654 According to International Patent Classification (IPC) or to both national classification and IPC														
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/479, 484, 652, 654  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Extra Sheet.														
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
Y	GOODMAN GILMAN, A. et al. The Pharmacological Basis of Therapeutics (6th Ed.). New York: Macmillan. 1980, pages 93-94 and 104-108, see entire document.	1-32												
Y	DIPIRO, J T et al. Pharmacotherapy: A Pathophysiologic Approach. New York: Elsevier. 1989, pages 153-157, see entire document.	1-32												
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"><tr><td>* Special categories of cited documents:</td><td>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr><tr><td>*A* document defining the general state of the art which is not considered to be of particular relevance</td><td>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr><tr><td>*B* earlier document published on or after the international filing date</td><td>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td></tr><tr><td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td><td>*A* document member of the same patent family</td></tr><tr><td>*O* document referring to an oral disclosure, use, exhibition or other means</td><td></td></tr><tr><td>*P* document published prior to the international filing date but later than the priority date claimed</td><td></td></tr></table>			* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family	*O* document referring to an oral disclosure, use, exhibition or other means		*P* document published prior to the international filing date but later than the priority date claimed	
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*P* document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 24 OCTOBER 1998		Date of mailing of the international search report 04 NOV 1998												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer M. MOEZIE  Telephone No. (703) 308-1235												



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/17680

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

CAPLUS, BIOSIS, EMBASE, MEDLINE, WPIDS, USPTAFULL- compounds or classes of compounds herein individually and together in compositions for the treatment of cardiac disorders or any other disorders.